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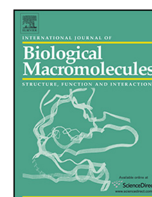

# Characterization of gelatin/chitosan scaffold blended with aloe vera and snail mucus for biomedical purpose

Daniel Enrique López Angulo*, Paulo José do Amaral Sobral

*University of São Paulo. Depto de Eng. de Alimentos – FZEA – USP, Av. Duque de Caxias Norte, 225, CEP, 13635-900 Pirassununga, (SP), Brazil*

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ABSTRACT

Biologically active scaffolds used in tissue engineering and regenerative medicine have been generating promising results in skin replacement. The present study aims to test the hypothesis that the incorporation of Aloe vera and snail mucus into scaffolds based on gelatin and chitosan could improve their structure, composition and biodegradability, with a potential effect on bioactivity. Homogeneous pore diameter as well as pore walls in the composite scaffold could be seen in the SEM image. The pores in the scaffolds were interconnected and their sizes ranged from 93 to 296 μm. The addition of Aloe vera and snail mucus enlarged the mean pore size with increased porosity and caused changes in the pore architecture. The FTIR analysis has shown good affinity and interaction between the matrix and the Aloe, which may decrease water-binding sites, so this fact hindered the water absorption capacity of the material. The mechanical properties could explain the highest swelling capacity of the snail scaffold, because the high percentage of elongation could facilitate the entry of liquid in it, generating a matrix with plenty of fluid retention. The real innovation in the present work could be the use of these substances (Aloe and snail mucus) for tissue engineering.

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## 1. Introduction

Acute trauma, genetic skin disorders and chronic wounds may result in skin loss, with burns and scalds being major contributors to rapid, extensive, deep (i.e. full thickness) wounds with substantial areas of skin damage, frequently without the possibility of skin regeneration [1]. These often cannot be successfully treated with common routine surgical skin grafting techniques [2,3]. The conventional therapies used to repair skin defects include autograft, allograft and xenograft. Although often there are limitations regarding donor sites and high risk of immune rejection and disease transmission [4,5].

In recent years, biologically active scaffolds used in tissue engineering and regenerative medicine have been generating promising results in skin replacement and cartilage regeneration. Many kinds of synthetic and natural polymer materials have been used as scaffolds. Their function is to provide mechanical support and to allow the adhesion and proliferation of the cells embedded on the scaffold or the cells migrating from a surrounding tissue [6]. A scaffold should be biocompatible, non-antigenic, biodegradable and have a three-dimensional structure with adequate porosity and pore size [7,8]. Gelatin is a biodegradable and biocompatible polymer and is able to make polyion complexes. Due to these properties, gelatin is commonly used in drug and cell delivery for tissue engineering applications targeting several tissues such as bone, cartilage and skin [9,10].

Among the numerous materials that have been evaluated for scaffold fabrication, in tissue engineering, chitosan is considered promising, attributing to its non-toxicity excellent biocompatibility and biodegradability [11]. Chitosan is a polysaccharide-type biological polymer composed of glucosamine and *N*-acetyl glucosamine linked with β 1–4 glucosidic linkage. Chitosan is generally produced by alkaline deacetylation of chitin. The *N*-acetyl glucosamine in chitosan is a structural component, also found in the glycosaminoglycans. This suggests that chitosan may be able to interact with growth factors, receptors, and adhesion proteins [12,13]. This polymer is found to be degraded by enzymes in the human body, and the degradation products are non-toxic [14].

In the context of incorporating new biomaterials that have regenerative and stimulating properties of cell growth, snail mucus has been used in medicine from ancient times for pain relief, for the treatment of burn injuries, other injuries and various diseases [15]. Research on the secretions of the snail (*Helix aspersa*) have

* Corresponding author.
*E-mail addresses:* daniel.lopez@usp.br, daniel.lopezan@usach.cl
(D.E. López Angulo).

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confirmed that the mucus contains an unusual combination of natural ingredients with beneficial and therapeutic qualities for human skin, including allantoin and glycolic acid [16,17]. According to the USA Food and Drug Administration (FDA), allantoin is a safe and effective active compound for skin protection at a dosage range of 0.5–2.0% [18]. Glycolic acid is the most widely used alpha-hydroxy acid for skin care cosmetic products and for the treatment of skin diseases including actinic keratosis, hyperkeratosis and seborrheic eczema [19].

Aloe vera (*Aloe barbadensis Miller*) is a member of the Liliaceae family. The parenchymatic tissue of Aloe vera leaves (gel) contains over 98–99% water and more than 60% of the dry matter (DM) is made up of polysaccharides [20]. Aloe vera gel is not only a hydrating and moisturizing agent for the skin, but it has also been reported to possess immunomodulatory, anti-inflammatory, anti-allergic, antibacteric and wound and burn healing properties [21–23]. There is a consistent number of reports regarding biologically active glycoproteins from Aloe vera [24]. Lately, Choi et al. [25] reported the proliferation and wound healing effect of a 5.5 kDa glycoprotein. This glycoprotein was isolated by activity-guided sequential fractionation from Aloe vera gel and was found to enhance keratinocyte proliferation.

Pore size, scaffold porosity, and the general pore structure all have significant effects upon tissue structure and infiltration into material constructs [26]. Therefore, Gelatin/Chitosan/Aloe vera (GCA) and Gelatin/Chitosan/Snail mucus (GCS)-based scaffold polymers may be combined to obtain a variety of biocompatible products for tissue engineering. Besides, there is no information related to their physical properties and skin generation properties. Porous chitosan/gelatin scaffold can be prepared via polyelectrolyte complex formation, freeze-drying, and post-crosslinking with glutaraldehyde. Glutaraldehyde is the most widely used crosslinker agent because it is easily available, inexpensive and capable of accomplishing the crosslinking in a relatively short time. Although other crosslinking agents could be used to reduce cytotoxicity, they cannot match glutaraldehyde in collagen stabilization. Although glutaraldehyde toxicity poses a risk for biocompatibility, it has also been shown that this can be reduced by decreasing the concentration of glutaraldehyde in the solution that generates the vapor, and/or by an efficient removal of unreacted glutaraldehyde left in the material after the crosslinking treatment [27].

Hence, this study aims to test the hypothesis that the incorporation of Aloe vera and snail mucus into scaffolds based on gelatin and chitosan could improve their structure, composition and biodegradability, with a potential effect on bioactivity, thereby marking this new biomaterial as a prospective scaffold candidate for tissue engineering. Finally, it is important to note that the real innovation could be the use of these substances (Aloe vera and snail mucus) for tissue engineering.

## 2. Material and methods

### 2.1. Materials

The macromolecules used for scaffold production were: pigskin gelatin (molecular weight $\sim 5.2 \times 10^4$ Da; bloom 260; moisture content = 9.98%), supplied by Gelnex South America (Itá, Santa Catarina, Brazil), and chitosan (derived from crab shell with minimum deacetylation degree of 85%, MW: $2 \times 10^5$), obtained from Sigma (St. Louis, MO, USA). Acetic acid (Sigma), PBS (Sigma Aldrich) and glycerol (Synth) were all chemical reagents of analytical grade. Aloe vera pure extract (A) was obtained by Mundo Aloe Vera Pica (Chile), snail mucus (S) was purchased from Lacofar LTDA (Chile).

### 2.2. Biochemical characterization

The concentration of the amino acids present in pigskin gelatin was determined by high-performance liquid chromatography (HPLC) following the methodology described by Díaz et al. [28].

### 2.3. Fabrication of porous scaffolds

A blend of gelatin (G) and chitosan (CH) was prepared by thorough mixing of gelatin (2%) and chitosan (1%) (control) solution in 0.05 M acetic acid in the ratio of 1:1 and stirred with a magnetic bar at 50 °C for 2 h. Similarly, Aloe vera (A) and snail mucus (S) blended G-CH composite was prepared by mixing different weighted quantities of A and S with the G-CH solution to obtain a final concentration of 0.07% (A1, S1, with low Aloe and snail mucus concentration), 0.15% (A2, S2, with high Aloe and snail mucus concentration) (w/v), and of both (AS 1 and AS 2, low and high concentration, respectively). Glycerol was added in 0.3%. The solution blend was then poured into a glass petri dish and left for 2 h on liquid nitrogen system chamber (−190 °C), prior to freeze-drying, at −58 °C, for 18 h. The sponge obtained was cross-linked inside desiccators containing glutaraldehyde (10%) in 200 ml 90% ethanol, during 2 h. Then, the sponge was immersed on NaOH solution (1%) and washed with distilled water two times, and treated with 5% (w/v) $NaBH_4$ solution. The scaffolds, again after thorough washing with distilled water, were frozen at −190 °C, and freeze-dried in vacuum for 18 h.

### 2.4. Microstructural observation

The morphology of the freeze-dried composite scaffolds was observed by using Scanning Electron Microscope (Hitachi TM 3000, Japan). The sample was placed on double-sided carbon tape in a vacuum chamber, prior to measurement. The pore sizes of the scaffolds were measured using image visualization software (Image J 1.45s, NIH Image, USA). The values were expressed as mean ± standard deviation.

### 2.5. Scaffold porosity

The porosity of scaffolds was measured by liquid displacement method, at 25 °C. Absolute ethanol was used as a displacement liquid since it can easily penetrate the scaffolds and would not induce shrinking or swelling. The specimens, pycnometer and ethanol, were kept at 25 °C for 1 h before testing. A pycnometer filled with ethanol was weighed (W1). A scaffold specimen of known weight (WS) was immersed into the bottle, and then the bottle was submerged slowly until all the air in the scaffold was removed; lastly the pycnometer was refilled with ethanol and weighed (W2). The scaffold saturated with ethanol was removed from the pycnometer and then the pycnometer was weighed (W3) [29]. Then, the volume of the scaffold (VS) specimen was calculated using Eq. (1), while the total volume of the pores (VP) was calculated using Eq. (2).

$$VS = \frac{W1 - W2 + WS}{\rho} \quad (1)$$

$$VP = \frac{W2 - W3 - WS}{\rho} \quad (2)$$

Then the porosity of the specimen was calculated using Eq. (3).

$$\xi = \frac{VP}{(VP + VS)} = \frac{W2 - W3 - WS}{W1 - W3} \quad (3)$$

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